Pseudomonas aeruginosa pneumonia: from microbial physiopathology to treatment

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February 2011
**Pseudomonas aeruginosa**

The facts:

- **opportunist pathogen**

- responsible for ~30% of nosocomial infections
  - 47% of ventilator associated pneumonia (VAP)
  - leading cause of bacteremia associated with high mortality (> 40%)

- therapeutic approaches are limited because of:
  - broad intrinsic antimicrobial resistance
  - its tendency to rapidly acquire resistance during antimicrobial therapies
# Impact of primary infection site on mortality

<table>
<thead>
<tr>
<th>Primary site</th>
<th>Cases</th>
<th>Mortality %</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown</td>
<td>58</td>
<td>13</td>
<td>-</td>
</tr>
<tr>
<td>Respiratory tract</td>
<td>24</td>
<td>55</td>
<td>0.03</td>
</tr>
<tr>
<td>Urinary tract</td>
<td>22</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>Line infections</td>
<td>5</td>
<td>0</td>
<td>ND</td>
</tr>
</tbody>
</table>

**Adjusted hazard ratio (95% CI)**

- Respiratory: 0.21, P=0.03
- Urinary/vascular: 0.01, 0.1, 1, 10

AAC 2003;47:2756
**P. aeruginosa and intubated patients**

Risk for colonization increases with time of intubation

10-20% of colonized patients develop *P. aeruginosa* VAP

30 - 40% mortality due to VAP
Are there microbiological determinants that influence the outcome of *P. aeruginosa* infections?

Is the expression of specific virulence determinants (phenotypes) associated with a worse outcome?
Major virulence determinants

Cytotoxicity
TTSS

Type IV pili

flagellum

Siderophores:
- pyoverdine
- pyochelin

Quorum sensing
- elastase
- phospholipase C
- lipase
- > 100 genes

rhamnolipids
- pyocyanin
- cyanide
- > 100 genes
Could outcome be linked to specific strains?

Type III secretion system

- 35 VAP isolates
  - 27 (77%) produced type III secreted proteins in vitro
    - 22 (81%): severe disease (death or relapse)
  - 8 strains didn’t produce type III secreted proteins
    - 3 (38%): severe disease (p<0.05)
  - 10 strains produced ExoU
    - 9 (90%): severe disease

VAP with isolates producing type III secretion-dependent exoproducts, especially ExoU, *in vitro* are associated with worse clinical outcome. However these studies didn’t analyze whether cytotoxicity is associated with infections.

*Crit Care Med 2002;30:521*
QS regulation in *P. aeruginosa*

QS controls expression of 200-300 genes (~5% of genome)

Adapted from Wade et al. J. Bacteriol. 2005
Inter-cellular communication

Allows a bacterial population to coordinate

Keller and Surette, Nat Rev Microbiol. 2006
QS essential for \textit{P. aeruginosa} virulence in...

- Plants (\textit{Arabidopsis})
  (Lettuce)
- Nematodes (\textit{C. elegans})
- Insects (\textit{Drosophila})
- Amoeba (\textit{D. discoideum})
- Mouse
- Human infections
Prospective study on P. aeruginosa colonization in the absence of antibiotic treatment

13 European ICUs: 31 patients

Daily tracheal aspirate

- one P. aeruginosa isolate
- total genomic DNA
- total RNA
- autoinducer

Colonization Infection

D1 D5 D10 D18 D25
intubation extubation
QS-proficiency and rhamnolipid production of initial colonizing isolates is associated with pneumonia in the placebo group.

- 57% of patients initially colonized by QS-proficient isolates versus 9% colonized by QS-deficient isolates developed VAP (P= 0.018)

- Production of the QS-dependent virulence factor rhamnolipids is associated with VAP (P= 0.003)

Thorax 2010;65:703
Role of rhamnolipids

- Uptake of hydrophobic molecules (1992)
- Surfactant for swarming motility (2000)
- Disrupt tight junctions in human airway epithelia (2006)
- Lyse PMNs *in vitro* (2007)
QS-deficient isolates (LasR mutants) increase during colonization

31 placebo patients

Days of colonization

% patients with Qs mutants

LasR

RhlR

PNAS 2009;106:6339
In patient population dynamics: one genotype

Isolate (in vitro)

<table>
<thead>
<tr>
<th>wt</th>
<th>ΔlasR</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

ΔlasR primer only detects wt
In patient population dynamics: one genotype

Isolate
in vitro

Population
in patient

Genomic DNA

In patient population dynamics: one genotype

... lasR mutants dominant in the population !!!
In patient population dynamics: two genotypes

... lasR mutants dominant in the population !!!

PNAS 2009;106:6339
**Bacterial social behaviours**

**Signal**: elicits response in recipient, induced response is beneficial for the actor

**Public good**: resource that is costly to produce and provides benefit to all individuals in the population

**Cooperation**: behavior that benefits another individual (recipient) and that is maintained because of its beneficial effect on the recipient

**Cheater**: individual who does not cooperate, but gain benefit from others cooperating
Why do *lasR* mutants outcompete wt?

Cooperator (ex: QS wild type isolate)

Public goods (ex: polypeptides, produced by elastase)
Quorum sensing as a social behavior

Cooperator (ex: QS wild type isolate)

Public goods (ex: polypeptides, produced by elastase)

Non-cooperator or cheater (ex: a lasR mutant)

elastase
Quorum sensing as a social behavior

Cooperator (ex: QS wild type isolate)

Public goods (ex: polypeptides, produced by elastase)

Non-cooperator or cheater (ex: a lasR mutant)

QS cheaters (lasR mutants) have fitness advantage BUT only in the presence of QS cooperators !!

PNAS 2009;106:6339
QS is important for development of VAP

- VAP occurs earlier in patients colonized by QS-proficient isolates
- Progressive accumulation of QS-deficient isolates might protect from VAP

PNAS 2009;106:6339
Antibiotic therapy and virulence factor production

**Patient A**

**Patient B**

**Patient C**

*Van Delden et al, unpublished results*

**Conclusion:**

1. Fluctuations of quorum-sensing dependent virulence factor production appear after discontinuation of antimicrobial therapies.
2. Antimicrobial therapies might select quorum-sensing proficient isolates.
Bacterial warfare: R-pyocin mediated killing

Landing

Drilling

Killing

relaxed

contracted

Core
R-pyocin warfare *in vivo*?

[Graph showing colonization rates and genomic copy percentages over days of colonization.]

Initial clone G is killed by clone L by R2 pyocin.

*J Bact* 2010;192:1921
Working model for R-pyocin – LPS interaction

Other serotypes: receptors may be the same, but shielding differs according to B-band charge and packaging
Summary

- Phenotype and NOT genotype associated with *P. aeruginosa* VAP
- Rhamnolipid production (*rhlR* QS system) high risk factor for VAP
- *P. aeruginosa* adapts to lung environment by mutation of *lasR*
  - Many patients co-colonized by wt and *lasR* mutants
  - *lasR* mutants: social « cheaters » or part of cooperative strategy?
  - one genotype: *lasR* mutant out-competes wild-type population
  - multiple genotypes: other factors such as bacterial warfare
determine population dynamics
How should we treat Pseudomonas infections?
Resistance of *P. aeruginosa* can be predicted

### Table 3. Multivariate association, averaged across antipseudomonal agents, of previous exposure to an agent, and resistance to that same agent in 267 bacteremic strains of *Pseudomonas aeruginosa*.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Adjusted OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous monotherapy with the agent</td>
<td>2.5 (1.3–4.8)</td>
<td>.006</td>
</tr>
<tr>
<td>Previous combination therapy including the agent</td>
<td>1.8 (0.55–5.8)</td>
<td>.34</td>
</tr>
<tr>
<td>Severe sepsis or septic shock</td>
<td>1.6 (0.94–2.6)</td>
<td>.08</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antipseudomonal agent, included in previous therapy</th>
<th>Resistance of the bacteremic strain to this agent</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (cases)</td>
<td>No (controls)</td>
<td>OR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>Yes</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>13</td>
<td>246</td>
<td>11.4 (1.6–64.7)</td>
</tr>
<tr>
<td>Piperacillin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Yes</td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>26</td>
<td>231</td>
<td>4.4 (0.67–22.1)</td>
</tr>
<tr>
<td>Imipenem&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Yes</td>
<td>11</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>30</td>
<td>186</td>
<td>2.7 (1.1–6.5)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>Yes</td>
<td>0</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>15</td>
<td>243</td>
<td>0.0 (0.0–9.1)</td>
</tr>
<tr>
<td>Aminoglycoside</td>
<td>Yes</td>
<td>6</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>37</td>
<td>198</td>
<td>1.2 (0.39–3.4)</td>
</tr>
</tbody>
</table>

**Conclusion:** preceding ceftazidime and imipenem exposure, especially as monotherapy, was associated with resistant *P. aeruginosa* bacteremic isolates
Evolution of antibiotic resistance

Patient A

First detection, VAP

6 days

Tobramycin

6 days

Pip-Taz

Tobramycin

Pip-Taz

Pip-Taz

0 5 6 7 20 23 26 30 36 43 47 50 54 57 68 71 76 78 92

Reinhardt et al., AAC 2007

Susceptible
Intermediate
Resistant

Pip., Cefta, Cefep., Imi., Mero., Pip. + Taz

Aztreo., Amika., Genta., Netil., Tobra.

Norflo.

Cipro.
Emergence and NOT acquisition of resistance
Pip-Taz resistance correlates with *ampC* expression
Evolution of antibiotic resistance

Patient B

First detection of *P. aeruginosa*

**RAPD type:** ba  a  b  cc  bb  bbb  bbb  bbb  bbb  b  b  b  b  b

**I and R isolates derive from isogenic susceptible parent**

Reinhardt et al., AAC 2007
Evolution of antibiotic resistance

Patient C

All isolates remain susceptible!
Major antibiotic resistance mechanisms

- Efflux pumps
  All classes of antibiotics

- AmpC β-lactamase
  Penicillins, cephalosporins

- Topoisomerases
  Quinolones

Porin OprD
  Carbapenem
# Dynamics of antibiotic resistance

<table>
<thead>
<tr>
<th>Pat.</th>
<th>treatment</th>
<th>mechanism</th>
<th>emergence</th>
<th>stability¹</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>imipenem</td>
<td>OprD</td>
<td>6 days</td>
<td>&gt; 80 days</td>
</tr>
<tr>
<td></td>
<td>pip/taz</td>
<td>AmpC</td>
<td>6 days</td>
<td>&lt; 7 days</td>
</tr>
<tr>
<td>B</td>
<td>ciprofloxacin</td>
<td>MexCD</td>
<td>10 days</td>
<td>&lt; 40 days</td>
</tr>
<tr>
<td></td>
<td>cefepime</td>
<td>MexXY</td>
<td>10 days</td>
<td>&lt; 15 days</td>
</tr>
<tr>
<td>C</td>
<td>amik+imi</td>
<td>none</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>cefep+tobra</td>
<td>none</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

¹ after treatment stop
NA, not applicable

Combination therapy prevented resistance emergence?
Is combination therapy better than monotherapy?

Combination therapy

• The pros
  ▪ Decreases the risk of an inappropriate empirical therapy
  ▪ Might reduce the risk of selection of resistant isolates
  ▪ The interaction might be synergistic and increase the killing

• The contras
  ▪ Higher costs
  ▪ More side effects
  ▪ Possibly higher risk of superinfection with fungi due to wider spectrum
Do meta-analyses help us?

β-lactam monotherapy versus β-lactam-aminoglycoside combination therapy for sepsis in immunocompetent patients: systematic review and meta-analysis of randomised trials
Mical Paul, Ishay Benuri-Silbiger, Karla Soares-Weiser, Leonard Leibovici

No advantage for *P. aeruginosa* bacteremia

Does combination antimicrobial therapy reduce mortality in Gram-negative bacteraemia?
A meta-analysis
Nasia Safdar, Jo Handelsman, and Dennis G Maki

Significant survival benefit for *P. aeruginosa* bacteremia
No compensation possible for:

- No evaluation of adequacy of the empirical and definitive therapies
- Inclusion of patients receiving aminoglycoside monotherapy
- Low number of documented *P. aeruginosa* bacteremia in each trial
Time to death during *P. aeruginosa* bacteremia

Conclusion: 16 of 45 (35%) patients who died did die within the first 5 days

**AAC 2003;47:2756**
Impact of mono versus combination empirical therapy on early deaths

**Conclusion**: empirical combination therapies do not improve the outcome of those patients that are so sick that they will die within the first days

AAC 2003;47:2756
Impact of mono versus combination definite therapy on late deaths

**Conclusion:** a definitive combination therapy does not improve the outcome

_AAC 2003;47:2756_
Impact of mono versus combination empirical therapy on late deaths

**Conclusion**: empirical combination therapy improves the outcome at 30 days after censuring for patients that die within the first 5 days

*AAC 2003;47:2756*
Antibiotic

Essential target:
- DNA replication
- Protein synthesis
- Cell wall synthesis

Selection for Antibiotic resistance

Classical antibiotics:

Anti-virulence strategies:

Anti-virulence molecule

Non-essential target:
- flagella (vaccine)
- virulence factor synthesis (QS)

Theoretically no selection pressure for resistance
Azithromycin is beneficial in CF patients colonized by *Pseudomonas*

Azithromycin improves FEV1, reduces acute exacerbations and increases weight gain in CF patients colonized by *Pseudomonas*
Azithromycin does not improve pulmonary function in the absence of *Pseudomonas*

The beneficial effect of azithromycin in CF patients is restricted to patients colonized by *Pseudomonas*.

*JAMA* 2010;303:1707
Azithromycin initiated before BOS Stage 2 is associated with reduced mortality in multivariate analysis

- Retrospective, 78 treated compared to 95 non treated

(J Heart Lung Transpl 2010;29:531)
Azithromycin increases BOS free survival
But not overall survival

\[ ERJ 2010, \text{ in press} \]
AZM decreases QS gene expression in vitro

Autoinducer

Expression of Autoinducer synthase gene

Minimal inhibitory concentration for *P. aeruginosa*: 128 mg/l AZM

Tateda et al. AAC, 2001
# Prophylactic azithromycin prevents VAP

Placebo group

<table>
<thead>
<tr>
<th>RHAM</th>
<th>PAO1</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;90%</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>90-10</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>&lt;10%</td>
<td>0</td>
</tr>
</tbody>
</table>

Azithromycin treatment has prevented 5 putative cases of VAP

| Patient | Score | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|---------|-------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|
| X/101   | 1.78  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| X/104   | 0.88  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| X/105   | 0.25  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| X/106   | 0.67  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Days of colonization

| Patient | Score | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|---------|-------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|
| X/101   | 1.78  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| X/104   | 0.88  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| X/105   | 0.25  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| X/106   | 0.67  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

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|---------|-------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|
| X/101   | 1.78  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| X/104   | 0.88  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| X/105   | 0.25  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| X/106   | 0.67  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| Patient | Score | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|---------|-------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|
| X/101   | 1.78  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| X/104   | 0.88  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| X/105   | 0.25  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| X/106   | 0.67  | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
Azithromycin inhibits “in patient” the production of QS genes

Plos Pathogens 2010; 6: e1000883
Inhibition of QS by azithromycin selects for persistent colonization with QS-proficient isolates

- Whereas placebo treated patients are progressively colonized by QS-deficient isolates, azithromycin treated patients remain colonized by QS-proficient isolates

*Plos Pathogens 2010; 6: e1000883*
Mechanisms of action of azithromycin on Pseudomonas infections

- Chronic colonization by *Pseudomonas* expressing virulence genes increases local inflammation potentially responsible for decrease in lung function seen in both CF and BOS

  - Azithromycin might have a direct anti-inflammatory effect

  - Part of the clinical benefit observed with azithromycin in CF and BOS might be due to an indirect anti-inflammatory effect due to inhibition of quorum-sensing dependent virulence of *Pseudomonas*